cells, T cells, endothelial cells, B cells, natural killer cells, megakaryocytes, eosinophils, and progenitors and progeny thereof, wherein said embryonic blast cell population can be derived by:

(a) culturing an embryonic stem cell population in an embryoid body cell medium comprising platelet-poor fetal bovine serum (PP-FBS) from about 3 days to about 4 days to obtain an embryoid body cell population; and

(b) culturing said embryoid body cell population in an embryonic blast cell medium comprising platelet-poor fetal bovine serum (PP-FBS) and at least one growth factor selected from the group consisting of a hematopoietic cell growth factor and an endothelial cell growth factor, from about 3 days to about 6 days to obtain a cellular population comprising embryonic blast cells.

REMARKS

Claims 36 and 57 have been canceled without prejudice or disclaimer of the subject matter therein. New Claim 107 has been added to more particularly describe the present invention. No new matter is added by Claim 107.

Objection to the Specification and Rejection of Claims 27-59 Under 35 U.S.C. § 112, first paragraph:

The Examiner has objected to the specification and rejected Claims 27-59 under 35 U.S.C. § 112, first paragraph, contending that the claims contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. Specifically, the Examiner contends that a cell line is required to practice the invention and that the cell line must be obtainable by a repeatable method set forth in the specification or otherwise be available to the public.

Applicants submit that the specification clearly sets forth a repeatable method by which the claimed embryonic blast cell population can be obtained, and that therefore, a deposit of the cell line is not required. Indeed, the method for predictably obtaining the embryonic blast cell population is one of the novel features of the present invention. More particularly, the specification goes to great lengths to specify the precise parameters which are required to produce the claimed cell populations and to avoid the failures of prior investigators. Finally, the starting materials are all readily available to the public and well known in the art.

As clearly set forth in the specification, an embryonic blast cell population of the present invention is obtained by the following culture conditions. First, an embryoid stem cell population is isolated and cultured to form embryoid body cell

population of the present invention. This procedure is described in detail in the specification on page 13, line 8, to page 17, line 11, and in the Example 1. The preferred medium, added factors, optimum temperatures, optimum culturing environment, optimum cell density, and time of culture are described in detail. Furthermore, the specification clearly emphasizes that factors which are important for isolating the embryoid body cell population of the present invention include: (1) the culture time, with from about 72 hours to 96 hours being most preferred, and (2) the use of platelet poor fetal bovine serum (PP-FBS) in the medium (i.e., FBS from which inhibitors of ES differentiation have been removed). Applicants submit that application of the culture parameters do not require skill beyond that of one of ordinary skill in the art and that by following the guidance in the specification, including the examples provided, an embryoid body cell population of the present invention can be repeatably obtained.

The second step in obtaining an embryonic blast cell population of the present invention is to culture the above-isolated embryoid body cell population under conditions effective to produce the claimed pluripotent embryonic blast cell population. Again, these culture conditions are clearly set forth in detail in the specification on page 25, line 11, to page 29, line 19, and in Example 3. Such culture conditions include (1) the use of PP-FBS for the EB culture media; (2) the addition of methyl cellulose to promote clustering of cells; (3) the addition of one or more growth

factors, preferably a mixture of hematopoietic and endothelial cell growth factors; and (4) a specified culture time, with about 3 to about 6 days being most preferred. Again, it is well within the abilities of one of ordinary skill in the art to use the guidance provided in the specification, including the working examples, to repeatably obtain the claimed embryonic blast cell population. Therefore, Applicants submit that the written description is sufficiently enabling to avoid the need for a deposit.

In view of the foregoing, Applicants respectfully request that the Examiner withdraw the objection to the specification and rejection of Claims 27-53 under 35 U.S.C. § 112, first paragraph.

Rejection of Claims 27-59 Under 35 U.S.C. § 102(b), or § 103(a):

The Examiner has rejected Claims 27-59 under 35 U.S.C. § 102(b) as anticipated by, or in the alternative, under 35 U.S.C. § 103 as obvious over Wiles et al. The Examiner contends that Wiles et al. disclose an embryonic blast cell population which appears to be identical to the claimed embryonic blast cell population. The Examiner further contends that even if the populations of Wiles et al. and the claimed populations are not the same, the reference preparations would have rendered the claimed population obvious to one of ordinary skill in the art at the time the invention was made in view of the close relationship between the cells.

Applicants traverse the Examiner's rejection of Claims 27-59. Before discussing the distinguishing features of the present

invention over the cited reference, Applicants note that Claims 27 and 43 have been amended in order to more clearly describe the claimed embryonic blast cell population which is Applicants' invention. Specifically, Claims 27 and 43 now recite that the embryonic blast cell population of the present invention is pluripotent for development into primitive erythroid cells, definitive erythroid cells, macrophages, neutrophils, mast cells, T cells, endothelial cells, B cells, natural killer cells, megakaryocytes, eosinophils, and/or progenitors and progeny thereof.

Prior to addressing the specific rejections, Applicants will generally address a point which is pertinent to the understanding of the novelty and nonobviousness of the claimed invention. Applicants have identified and isolated for the first time, a pluripotent, embryonic blast cell population which is the earliest hematopoietic precursor to be isolated at the time of the present invention. This pluripotent precursor is distinguished from any other embryonic blast cell population identified in the art (including those which have been called "pluripotent"), because it is capable of developing into both the primitive erythroid lineage and all other hematopoietic lineages. Moreover, the novel method for isolating such a precursor cell was not appreciated by prior investigators, despite the desire in the art to obtain such a cell population. Therefore, the embryonic blast cell population of the

present invention is completely novel and nonobvious over the prior art.

The novel embryonic blast cell population of the present invention can be derived by culturing a novel embryoid body cell population of the present invention under specific conditions described in the specification. These culture conditions enable the isolation of the claimed embryonic blast cell population. More particularly, Applicants have discovered that culturing an embryonic stem (ES) cell population for a certain period of time under specific culture conditions results in the differentiation of the ES cell population into a substantially homogeneous embryoid body (EB) cell population in which the EB cells are pluripotent. This EB population can then be cultured under further specific conditions for a certain period of time to obtain the claimed embryonic blast cell population (as discussed in the section above).

As discussed above, an identifying feature of this pluripotent population of embryonic blast cells which distinguishes it from any previously described embryonic blast cell population is that the population of the present invention comprises a common, multipotential precursor that is capable of differentiating into primitive erythroid cells, and into other hematopoietic and endothelial cell lineages. Prior to the present invention, the relationship between primitive erythrocytes and other hematopoietic lineages was not known, and no embryonic blast cell precursor had

been isolated which could differentiate into both primitive erythroid cells and other hematopoietic lineages. A reason for the failure of prior investigators to isolate a common multipotent (i.e., pluripotent) precursor is that prior investigators failed to recognize a method for isolating substantially homogeneous populations of totipotent or pluripotent embryonic cells. Prior to the present invention, ES cells were cultured under conditions in which the cells committed to a cellular lineage early in the tissue culture process (i.e., the EB cell population lost pluripotency). The present inventors are the first to teach the culture conditions which maintain an EB cell population and an embryonic blast cell population at a stage of pluripotency.

Applicants submit that the pluripotent embryoid body cell population of the present invention and the embryonic blast cell population derived therefrom represent the earliest stage of embryonic hematopoietic commitment which has been isolated or described as of the time of the present invention.

With regard to the cited publication of Wiles et al., Applicants respectfully point out that Wiles et al. do not teach the specific culture conditions which result in the isolation of the novel embryonic blast cell population of the present invention. As described on page 260, "Differentiation conditions", of Wiles et al., embryonic stem cells were cultured directly in methyl cellulose media which did not contain platelet-poor fetal calf serum. The different culture conditions of the present invention

have been discussed in detail above. Wiles et al. also do not appreciate the importance of the timing of the culture as described in the present invention. As a result, the first cell population to be identified by Wiles et al. after the beginning of culture of the ES cells is the established erythroid lineage at 7-8 days, followed by the established macrophage lineage at 12-18 days (page 261, second column). In fact, the only other cell populations identified in Wiles et al. are neutrophils and mast cells. Furthermore, Wiles et al. do not identify any common precursor which can differentiate into each of these cell types. Wiles et al. failed to identify an embryonic blast cell population that, when plated into methyl cellulose cultures, resulted in the differentiation into colonies of primitive erythroid populations together with colonies of multiple other hematopoietic lineages including definitive erythroid, multilineage, and myeloid populations (See specification, Example 5). The culture conditions of Wiles et al. fail to isolate or identify a single pluripotent precursor population which is capable of differentiating into primitive erythroid cells and many other hematopoietic and endothelial lineages. Therefore, the claimed invention is not anticipated by Wiles et al.

With regard to the Examiner's contention that it would have been obvious to isolate the claimed population because the claimed cell lines are closely related, Applicants submit that the failure of Wiles et al., as well as any other prior investigator, to

identify and/or isolate a common multipotential precursor that can differentiate into both the primitive erythroid lineage and other hematopoietic and endothelial lineages is attributable to the failure of these investigators to appreciate the specific culture conditions by which such a population can be obtained. Applicants submit that the mere supposition that such a cell population might exist does not render the isolated claimed cell population obvious, particularly in view of the failure of others to identify and isolate such a population, despite years of trying to do so. Prior to the present invention, there was a long felt need in the art to isolate pluripotent hematopoietic precursor lineages, but until the present invention, no one has been able achieve this goal. Indeed, on page 266 of Wiles et al., Wiles et al. appear to acknowledge that repopulating hematopoietic stem cells and lymphoid precursors have not been identified or isolated, and that the identification of such cells may be difficult (column 2, second full paragraph). Applicants submit that it is well known and accepted in the art that the isolation of a particular cell lineage does not make it obvious to isolate the precursor lineage to that cell, and certainly not a precursor which may be several stages earlier. In addition, until the isolation of the novel embryonic blast cell population by the present inventors, it was not even known that primitive erythroid lineages and other hematopoietic lineages shared a common precursor. Therefore, Applicants submit that the claimed invention is not obvious over Wiles et al.

In view of the foregoing, Applicants respectfully request that the Examiner withdraw the rejection of Claims 27-59 under 35 U.S.C. § 102(b) and § 103.

Applicants submit that all pending claims are in condition for allowance and request the Examiner's favorable consideration and allowance thereof. Applicants have tried to respond to all issues raised by the Examiner in the April 29, 1997, office action. Applicants' attorney requests the courtesy of a telephone call from the Examiner in the event any of the claims are not considered to be in a condition for allowance.

Respectfully submitted,

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